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Timing manipulations reveal the lack of a causal link across timing of annual-cycle stages in a long-distance migrant

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Summary statement: Via experimental manipulations we show that pied flycatchers do not adjust the timing of their moult and migration to the termination of breeding.

Abstract

Organisms need to time their annual-cycle stages, like breeding and migration, to occur at the right time of the year. Climate change has shifted the timing of annual-cycle stages at different rates, thereby tightening or lifting time constraints of these annual-cycle stages, a rarely studied consequence of climate change. The degree to which these constraints are affected by climate change depends on whether consecutive stages are causally linked **(I)** or whether the timing of each stage is independent of other stages **(II)**. Under **(I)**, a change in timing in one stage has knock-on timing effects on subsequent stages, whereas under **(II)** a shift in the timing of one stage affects the degree of overlap with previous and subsequent stages. For testing this we combined field manipulations, captivity measurements and geolocation data. We advanced and delayed hatching dates in pied flycatchers (*Ficedula hypoleuca*) and measured how the timing of subsequent stages (male moult and migration) were affected. There was no causal effect of manipulated hatching dates on the onset of moult and departure to Africa. Thus, advancing hatching dates reduced the male moult-breeding overlap with no effect on the moult-migration interval. Interestingly, the wintering location of delayed males was more westwards, suggesting that delaying the termination of breeding carries-over to winter location. Because we found no causal linkage of the timing of annual-cycle stages, climate change can shift these stages at different rates, with the risk that the time available for some become so short that this will have major fitness consequences.

Keywords: European pied flycatcher, *Ficedula hypoleuca*, experiment, migration, moult, reproduction

Introduction

Organisms living in seasonal environments, such as temperate zones, are generally exposed to predictable changes in environmental conditions that determine the organization of their annual-cycle stages. Since some of these stages, such as breeding and migration, are energetically costly, their occurrence needs to be timed correctly to match favourable conditions (Enright, 1970; Gwinner, 1996; Jacobs and Wingfield, 2000; Wingfield, 2008). Annual-cycles vary in complexity in a species-specific way: from simple breeding/non-breeding transitions to the inclusion of other energetically demanding stages like migration or moult (Wingfield, 2008). As the total time available for the allocation of these seasonal stages is one year, more complex annual-cycles will necessarily involve a concomitant increase in time-constraints. This is especially true if these different stages are all energetically demanding and need to be temporally segregated (Dietz et al., 2013). Complex annual-cycles would thus result in a low flexibility as further shortening or overlapping stages would lead to fitness costs (Jacobs and Wingfield, 2000; Wingfield, 2008).

To achieve a synchronisation between annual-cycle stages and environmental cycles, organisms use predictive cues, with photoperiod and temperature as the most relevant ones (Dawson, 2015; Wingfield, 2008; Winkler et al., 2014). While photoperiod is important as a long-term predictive cue, since it remains unchanged across the years (Gwinner, 1989), temperature provides short-term temporal information for fine-tuning timing within a particular year and allows organisms to match their timing with the year-to-year variation in optimal timing (Dawson, 2005; Schaper et al., 2012; Visser et al., 2004; Visser et al., 2009). The importance of photoperiod and temperature as cues varies across annual-cycle stages. Hence, increasing temperatures due to climate change will cause shifts in timing of stages that are temperature sensitive, such as reproduction (Knudsen et al., 2011; Parmesan, 2006; Visser and Both, 2005), while other stages may not shift if mainly affected by photoperiod or circannual rhythms (Both and Visser, 2001; Tomotani et al., 2018a; Visser et al., 2004). How this affects the whole annual-cycle, however, depends on how stages are interrelated (Crozier et al., 2008; Hera et al., 2013).

One possibility is the existence of a tight link between stages. In this scenario (**scenario I: Linked**) the timing of a given stage depends strongly on the timing of the previous one(s). Such temporal links across

stages could be caused, for example, by pleiotropic effects of hormones regulating the transitions between stages (Dawson, 2006; Jacobs and Wingfield, 2000; Williams, 2012). This would mean that even if one stage is sensitive to temperature and the other not, shifts in a given stage would cause similar shifts in the subsequent ones. For instance, in an experiment with captive juvenile Eurasian blackcaps (*Sylvia atricapilla*), migratory activity was only initiated after the completion of moult, thus the interval between the two stages was fairly constant among individuals (Pulido and Coppack, 2004). Under this scenario, climate change would not increase or decrease the overlap of stages. However, depending on the magnitude of the shift, stages could be displaced in time and desynchronised from the optimal environmental conditions for that stage.

Alternatively, consecutive stages may not be linked (**scenario II: Independence**). Thus, if the effect of cues varies between stages, climate change will lead to shifts in timing of annual-cycle stages with different rates, causing an increase or decrease in the degree of overlap between events. This could result in time constraints being tightened or lifted throughout the annual-cycle because a shift in one stage would lead to more time in one part of the cycle, but subsequently may result in less time available for other stages. For example, between 1980-2000 European pied flycatchers (*Ficedula hypoleuca*) advanced egg-laying dates more strongly than their arrival dates at the breeding grounds, associated with climate change. This has led to a shorter interval between arrival and breeding, constraining how much laying dates could continue to advance with increased climate change (Both and Visser, 2001; Tomotani et al., 2018a).

Finally, it is also possible that both of the two above-mentioned scenarios occur in different parts of the cycle (Salis et al., 2017; Tomotani et al., 2016). For example, it has been suggested that pre-breeding stages are more time selected than post-breeding stages, and are, as a result, less plastic (Conklin et al., 2013; Karagicheva et al., 2016). Under that perspective, it would be expected that pre-breeding stages are more likely to fall under scenario II than post-breeding stages. Because these two scenarios have very different consequences in terms of costs of climate change, understanding annual-cycle adaptation to environmental changes need to incorporate knowledge on how consecutive stages are temporally linked.

This interrelation across annual-cycle stages is usually investigated by correlating patterns of timing and duration of annual-cycle stages at the individual (Conklin and Battley, 2012; Echeverry-Galvis and Hau, 2013; Gill et al., 2014; Low et al., 2015) and population levels (Both and Visser, 2001; Finch et al., 2014; Gill et al., 2014; Moyes et al., 2011; Ozgul et al., 2010; Rakhimberdiev et al., 2018). Such studies are necessary to describe when in the annual-cycle individuals may be shifted, constrained by or suffer from carry-over effects. However, to fully understand the temporal links across stages it is necessary to experimentally test for causality of these interrelations, thus controlling for potential confounding effects. A key way of doing this is to manipulate the timing of a given stage and look for cascading effects on the timing of consecutive stages.

A number of studies has directly or indirectly tested the link between consecutive stages, particularly by looking at the trade-off between moult and breeding (see Tomotani et al., 2018b for a revision). Many times the purposes of those studies was to understand how a costly breeding would cause a delay on the onset of moult, for example via food availability manipulations (Siikamäki, 1998) and experimental delays on timing of breeding (Hemborg, 1998; Nilsson and Svensson, 1996; Siikamäki, 1998), where delays lead to a higher moult-breeding overlap. The most comprehensive study that looked into the relationship between timing of breeding and timing of moult was Hemborg (1998). By manipulating hatching dates, he showed a different control for the moult onset of males and females: the manipulations did not affect female moult (consistent with scenario II), while it had an effect for males (consistent with scenario I). One limitation of the study is the use of moult scores instead of actual dates when moult started. This makes it more difficult to assess if individuals really started to moult at a certain chick age or calendar day (true scenario I versus II).

Here, we tested whether experimental shifts in the termination of breeding, as a key annual-cycle stage, causally affected the timing of subsequent stages. We experimentally shifted hatching dates as a proxy for the termination of reproduction and measured the timing of post-breeding moult and migration. Previous studies have suggested a difference in control for timing of moult of females and males, which leads to a sexual conflict since males and females unequally prioritize their current reproductive success (Hemborg, 1998; Hemborg, 1999; Hemborg and Merila, 1998; Tomotani et al., 2018b). Here we were

mostly interested in how adult males were affected. Because they supposedly invest less in their current reproduction, males present a larger moult-breeding overlap than females. Moreover, males have a higher fidelity to the breeding area (Both et al., 2016), making them more appropriate to measure the effects of our manipulations. Thus, after the chick-rearing phase, we monitored the males' post-breeding moult by temporally keeping them in captivity, their moult control via analyses of the hormone prolactin and, after release, the timing of their autumn migration and wintering site selection using light-level geolocators.

The timing of moult and departure could **(I) depend on a previous stage (Linked)**. Such previous stage could be: **(Ia)** termination of breeding, the manipulated stage, thus shifting the termination of breeding also shifts the onset of moult and migration; or **(Ib)** spring arrival or onset of breeding which are non-manipulated stages. Alternatively, **(II) timing does not depend on a previous stage (Independence)**. It could, for example, simply depend on photoperiod or other(s) cue(s), but not on the timing of breeding. Notice that in both **scenarios Ib** and **II** the timing of moult and migration departure will not be related to the manipulation, but, while between-year variation in the timing of moult and migration will certainly exist for **scenario Ib**, this will not necessarily be the case for **scenario II** (*i.e.*, if only related to photoperiod). Thus, we cannot completely distinguish between **Ib** and **II** as we would need more than two years of experiment to estimate year to year variation, but we can provide contrasting predictions for **scenario Ia** versus **Ib** and **II**.

Material and Methods

Study area and study species

European pied flycatchers are small long-distance migratory birds that reproduce in Europe and Western Asia and winter in West Africa (Ivory Coast in the case of this study population). Due to their acceptance of artificial nest-boxes and low nest desertion rates, it is possible to conduct field experiments and precisely monitor their breeding success. Voucher material of this study population was deposited in the

ornithology collection of the Naturalis Biodiversity Center (Leiden, The Netherlands) under the inventory numbers RMNH 592347, RMNH 592348 and RMNH 592349.

Fieldwork was conducted during 2013 and 2014 in the Hoge Veluwe National Park (The Netherlands; 05°51'E, 52°02'N). Forested areas in the park are dominated by pedunculate oaks (*Quercus robur*), the exotic northern red oaks (*Quercus rubra*), Scots pines (*Pinus sylvestris*), larches (*Larix* sp.) and birches (*Betula* sp.). We provide around 400 nest boxes year-round in an area of 171 ha., which are occupied in spring by cavity-nesting passerines such as pied flycatchers, great tits (*Parus major*), Eurasian blue tits (*Cyanistes caeruleus*), nuthatches (*Sitta europaea*) and coal tits (*Periparus ater*). We collect long-term field data on arrival dates of males, nest building of females, female egg-laying dates, chick hatching dates, brood success and adults and chick basic biometrics.

In order to place our results into an ecological context, we note that 2013 was a cold spring (April-June average temperature 11.25°C, against 12.65°C for 1995-2015), with a low density of caterpillars (average 2.4g biomass m^{-2} day^{-1}, against the 14.18 for 1995-2015) and peak on May 30th. The average flycatcher laying date was May 9th and the average number of fledgelings 5.78 (excluding manipulated broods), being above average (5.6 for 1995-2015). In contrast, 2014, was a warm spring (13.21°C), also with a low caterpillar density (0.67g biomass m^{-2} day^{-1}) but with a peak on May 3rd. The average flycatcher laying date was on May 6th and the average number of fledgelings 4.79 (excluding manipulated broods), thus, below average.

Foster pairs used to incubate experimental eggs (see below) consisted of naturally nesting great and blue tits found in the nest boxes of the Hoge Veluwe or in Heikamp, the Netherlands, a nest box population adjacent to the Hoge Veluwe.

Experimental design

Our experiment involved advancing and delaying the hatching date in two sets of breeding pairs, using a third set as control. To test for causal effects of shifting reproduction on the subsequent stages, we did not just use the natural variation in hatching dates. Instead, we incubated first laid eggs before the natural

incubation time and delayed incubation of later laid eggs after the natural incubation time. This resulted in an advancement and delay of hatching before and after the natural range of hatch dates.

All nest-boxes in the study area were checked twice per week until we found pied flycatcher nest material. We then inspected the nests daily to determine the date the first egg was laid. When we found three or more nests with first eggs laid on the same day, we randomly assigned them to a treatment (or randomly excluded them from the experiment, in case we had sufficient experimental groups or there were not enough nests to create additional groups of three nests).

In order to create differences in chick hatching dates, our treatments consisted of a combination of manipulating the start of the egg-incubation date, and thereby hatch date, and a split brood design (Fig. 1). We took advantage of the fact that these birds lay a single egg per day and usually start incubating their eggs only when the last one is laid.

One day after finding the first egg, when the three females had laid their second egg, we collected all these six eggs replacing them by plastic dummy eggs to prevent desertion. This clutch of six eggs (the typical clutch size for a pied flycatcher) composed of two eggs from all three nests was then placed under an already incubating great or blue tit (the "foster incubator") – therefore the initiation date of the incubation of this clutch was four days earlier than normal. We continued collecting the remaining eggs daily until the sixth egg was laid, always replacing them by dummy eggs. When the sixth egg was laid, we considered the clutch as complete. In case a female laid a seventh egg, the egg was removed from the experiment. All these newly collected eggs (lay order three to six) were stored in a field shed at ambient temperatures and turned twice per day to prevent damages to their viability (Visser and Lessells, 2001).

Once the female assigned as "control" laid her sixth egg, we swapped all plastic dummy eggs by a clutch created from eggs with lay order four and five. Because we gave the female six eggs on the same day that she laid her sixth egg, we did not modify the time when she started incubating or the hatching date of the eggs (Fig. 1). Thus, the birds hatched their chicks at the expected time. Eventual seventh eggs were removed as explained above.

As soon as the female assigned as "advanced" started incubating her dummy eggs continuously (*i.e.* when we found the female at least three times in a row incubating the dummy eggs within a few hours interval), which happened between one to three days after the last egg was laid, we replaced the dummy eggs by the six pre-incubated eggs (previously transferred to a foster incubator as described before). As mentioned above, because most pied flycatcher females typically start incubating their clutch once completed (six eggs), we advanced the hatching of these eggs by four days (Fig. 1). Thus, these birds hatched their chicks four days earlier than expected.

The females assigned as "delayed" only received a clutch of six eggs (with lay order three and six), replacing the dummy eggs, five days after the female laid the sixth egg (on the hypothetical "egg day 11"). This means that the starting point of incubation of these eggs was four days later than normal (Fig. 1). Thus, delayed birds hatched their chicks four days later than expected.

The procedure was repeated throughout the season as we tried to span the whole range of egg-laying dates as long as we found three females that laid their first egg on the same day. In a few cases (three nests in 2014) we allowed one day difference between nests to be able to create enough groups. In total, we managed to successfully manipulate 117 broods: 57 in 2013 (19 experimental groups) and 60 in 2014 (20 experimental groups). In five broods in 2013 and five in 2014, females laid five instead of six eggs, clutches of experimental groups involving those broods were completed by adding one of the occasional seventh eggs collected throughout the experiment (see above) - note that this procedure did not alter the timing treatment to the adult birds just the composition of egg origins in the brood. Due to desertions at incubation or chick stage, not all manipulations were successful. Whenever possible (four nests in 2013 and two in 2014), deserted manipulated clutches were moved to new pied flycatcher nests with maximum one day of laying date difference from the original experimental nest –this procedure also did not affect the timing treatment to the adult birds. Thus, the real starting number of manipulated broods was 111: 55 (18 advanced, 18 control and 19 delayed) in 2013 and 56 in 2014 (19 advanced, 18 control, 19 delayed). It is important to notice that eight days is a substantial difference in chick hatching dates for our birds. Average standard deviation of chick hatching dates per year of the entire population is just 5.2 days (mean for all years 1964-2012).

The number of hatched chicks did not differ across treatments (χ22,n=111 = 0.23, p-value= 0.89, advanced = 1.68 ±0.07, control = 1.72 ±0.07, delayed = 1.71 ±0.07), but we did not allow differences in brood sizes of more than one chick as this would affect the parental effort. Therefore, we checked all nests with two-days old chicks and, in case of nests with less than five hatched chicks (seven nests in 2013, four in 2014), we added extra chicks with maximum one day difference from nearby woodlands (e.g., other field sites like Westerheide, 5°50′E, 52°00′N, the Netherlands), raising the brood size to five chicks. Again, this procedure did not alter timing treatments, only the composition of sibling chicks in a nest. Treatments did not differ on the number of two-day old chicks (neither before, nor after the brood size correction).

Later during the experiment, we lost three nests (2013) due to natural causes (death of chicks, adult desertion at a later breeding stage or predation events) and not all males could be caught at either chick day seven (three males in 2013, three in 2014) or 12 (seven in 2013 and three in 2014). Therefore some variation existed in sample sizes across the different analyses and these are reflected in the degrees of freedom for each analysis.

Although only females incubate in this species, our treatment was effective in manipulating both sexes, as males and females are involved in chick rearing and thus the termination of breeding was modified for both sexes. Moreover, because the chick hatching dates was literally moved in time our manipulations were also useful to test effects of hatch dates on components of individual chick timing (Tomotani et al., 2016) and fitness.

Data collection

a) Field

The arrival date of individual males was assessed by daily identifying newly arriving males in the study area from early April until early May, using individual plumage characteristics and colour ring combinations (Both et al., 2016; Tomotani et al., 2017; Visser et al., 2015). Once a pied flycatcher pair

settled in a nest box and started building the nest, these were checked frequently to assess the exact date of egg-laying, clutch size, the exact date of chick hatching and number of hatched chicks.

After the manipulations took place, the breeding adults were caught when chicks were seven and then when they were 12 days old and their primary moult (henceforth "moult") was scored in both cases. Moreover, in order to obtain a more precise moult data, males were not released after being caught on chick-day 12 but taken to the Netherlands Institute of Ecology (NIOO-KNAW, Wageningen; see housing conditions below). Females were left in the field to continue to care for the chicks. In 2014 a subset of the chicks was also taken to the institute for a complementary study (Tomotani et al., 2016) and females from those nests were also taken to the institute.

b) Housing conditions

When in the laboratory, birds were housed in individual cages (90 cm × 50 cm × 40 cm) and provided food (mealworms, crickets and flies) and water *ad libitum*. Conditions of light and temperature were kept as close to the natural situation as possible, with all windows open, allowing birds to be exposed to natural illumination. This was supplemented by two fluorescent lamps connected to a timer that switched on 30 min after the sunrise and off 30 min before the sunset. Therefore, birds were exposed to the natural photoperiodic variation with natural duration of twilights. After 14 days in captivity, they were returned to the Hoge Veluwe and released close to their breeding nest box.

c) Timing of moult

In 2013, adults were further checked for moult after seven and 14 days of arrival in the laboratory (chick age 19 and 26 days). In 2014, however, moult was scored every day in most of the birds so the date the first feather was dropped could be pinpointed in most occasions. Moult score was based on which primary feather(s) was (were) being moulted and how much it had grown (following Dawson and Newton, 2004, but discriminating between old and dropped feathers). The score was then converted to the mass of newly grown feathers, which was assumed to increase linearly through time (Dawson and

Newton, 2004). Moult onset was then calculated by regressing a line through the different measurements of grown feather mass in different days. We used the method described in (Dawson and Newton, 2004) for calculating moult onset based on the feather mass grown and the R "moult" package (Erni et al., 2013), with the formula developed by (Underhill and Zucchini, 1988) to estimate the mass of growing feathers for each individual bird. Because we also had the dates that the birds lost their first primaries for most birds in 2014, we could compare data from the calculated and observed onsets. The calculated onsets differ from the observed first moult days by an average of 0.4 days (maximum 2.3 days); therefore, we are confident that our calculated moult onsets are accurate. In case we only had one day with a measurement of new feather mass (for example, a bird that was only observed moulting on the last time it was caught in the field), we first calculated the mean value of moult speed for the year (based on the slopes of the linear regression for all other individuals) and then used those calculated mean values of moult duration as a slope in the linear regression together with the feather mass to calculate the onset of moult for that individual. This assumes that there is little variation in the speed of moult among individuals which is not necessarily true as late moulting birds moult faster (Dawson, 2004). However, we assume this was a minor issue for our calculations, since **(a)** the number of birds affected was fairly small (two out of 49 moult data in 2013 and three out of 54 in 2014), and **(b)** those individuals affected did not have consistently very early or very late laying dates.

To assay the internal state of the animals, in 2013 we also performed an additional blood sampling for hormones. Prolactin is a hormone that could be involved in the transition between moult and breeding (Dawson, 2006), mediating the stress responses during breeding and having a role in the trade-off between parental effort and self-maintenance (Angelier and Chastel, 2009). Prolactin levels decline towards the end of the season, being lower when breeding is over and a decline in prolactin is expected to trigger moult onset (Dawson, 2006). We expected that, if animals adjusted their moult in relation to the termination of breeding, then we would also observe a shift in their prolactin levels, since we would expect that prolactin would be dependent on chick age (older chicks, lower values). Because birds were sampled at the same chick age, we would thus expect that that advanced, control and delayed birds from the same group (same lay date) would have the same prolactin level and that it would decline over time

equally for all treatments (similar to scenario 1b). In 2013 all males taken to the laboratory were blood sampled on the day they were captured (chicks of 12 days old). We collected 100 microliters of blood from the jugular vein of each individual as soon as the bird arrived at the laboratory, to prevent any effect of captivity stress on prolactin levels (Angelier and Chastel, 2009). Samples were immediately centrifuged and the plasma was collected and stored at -20° C. Samples were then shipped on dry ice to the Roslin Institute and levels of plasma immunoreactive prolactin was determined using a radioimmunoassay for recombinant-derived European starling (*Sturnus vulgaris*) prolactin described by (Bentley et al., 1997). All samples were measured in duplicate in a single assay. The sensitivity of the assay was 0.3 ng/mL, and the intra-assay coefficient of variation of this assay was 7.6%, and serial dilution of individual samples ran parallel along the standard curve within the dilution range assayed.

d) Timing of migration

Before being released back to the field, a subsample of the males (38 out of 42 in 2013 and 30 out of 50 in 2014, including individuals from all treatments across the whole range of laying dates), was deployed with 0.5 g light-level geolocators (Intigeo-W50, Migrate Technology Ltd, Cambridge, UK) using a leg-loop harness build with an 0.7 mm elastic nylon cord and adjusted to each bird with a knot in the posterior mounting tube, between the device's mounting loops.

Geolocators were recovered in the subsequent years (12 from the 2013 cohort: 10 in 2014, two in 2015; seven from the 2014 cohort: six in 2015, one in 2016) when the birds returned to breed. Data were processed similarly to Åkesson et al. (2012), but due to the imprecision of latitude data, we only used the information collected for longitude (inferred from local solar noon/midnight). Twilight transitions were determined using TransEdit (British Antarctic Survey, Cambridge) with a single threshold value of five, minimum daylight periods of one hour and minimum night period of four hours. Positions were obtained using the software BirdTracker (British Antarctic Survey, Cambridge), which gave us two positions per day (noon and midnight). Data were then visually inspected to detect large changes from the study area longitude, indicating a departure from breeding grounds, since pied flycatchers move to

the west, following the African coast during autumn migration (Ouwehand and Both, 2016; Ouwehand et al., 2016).

Effects of logger clock drift on longitude were tested as described in Ouwehand et al. (2016). There was no noticeable clock drift effect (always <1 min) and treatments did not significantly differ in terms of clock drift. In any case, we decided to use the average longitude of the first winter month (December) to extract the wintering longitude as the drift supposedly gets larger the longer the time span after deployment.

e) Fitness components

To assess the effects of our experiment on fitness, we measured breeding success and adult and chick condition (body mass) at the end of breeding. As a second metric of male condition, we also measured the quality of the feathers moulted after the experiment and collected in the subsequent year because a constrained moult is expected to lead to poor feather quality (Echeverry-Galvis and Hau, 2013; Vágási et al., 2012). Finally, we also obtained data on parental care terms of number of visits to the nest by each parent.

To measure breeding success, nests were regularly checked until all chicks fledged to obtain information on number of fledged chicks and overall brood success/failure.

To assess adult and chick condition, we took biometric measurements in the field of males, females and chicks. When chicks were 7 days old both adults were caught and we took measurements of tarsus length and weight. Later, when chicks were 12 days old, males and females were caught again and weighted for a second time. Adults and chicks were also blood sampled for paternity analysis (Tomotani et al., 2017).

To assess feather quality, in the years following the experiment (2014 and 2015), we sampled the fourth innermost primary of males that were in the experiment in the previous year, thus, the primary feather grown immediately after the experiment. We estimated the bending stiffness of feathers (miliNewton/mm) by testing the force necessary to bend the feather by applying a load at 32 mm from

the base of the rachis. We moved the load down at a speed of 4 mm/min and took the first 60 (mass-time) points immediately after contact of the load with the feather (1 point per second). Bending stiffness was estimated for each feather based on the slope of the relationship between feather mass and time (de la Hera et al., 2010).

To measure parental care, we fitted transponders with a unique individual code to each of the adults when their chicks were 7 days old and installed transponder readers at the entrance of each box to continuously measure the visits of each parent. To avoid disturbing too much the nest in one day readers were only installed when chicks were 8 days old and then removed when chicks were 12 days old and parents were caught for the second time. Thus, we only used transponder data made when chicks were 9, 10 and 11 days old. We collected data in most nests of 2013 (43 nests - due to a failure in some equipment and nest desertions at an early stage) and of a subsample of the nests in 2014 (6 nests).

The readers detect the presence of the transponder in the vicinity of the nest box every second. This means that if a bird is perched in the front of the nest box, the equipment will detect multiple records of this individual, even though it is not visiting the nest box multiple times. In order to filter out these pseudo visits, in 2015 we recorded transponder data together with video for another experiment (Tomotani et al., 2018b) and used the video data to discriminate which intervals actually reflected real visitations to the nest. We then used this information to clean up the data from 2013 and 2014. We are aware that year-to-year differences may exist, but reader configuration was kept constant across the 3 years, meaning that those differences should be relatively small.

f) Carry-over effects

In order to assess longer-term effects of our manipulations (as reversible state effects, Senner et al., 2015), we looked at the probability of finding the manipulated birds the following years as well as the breeding dates of males and females in the following year.

Data analysis

a) General

All analyses were performed in R version 3.2.1 (R Core Team 2015).

Most of the females were not taken to the laboratory (eight females taken to the lab) and were not moulting on the last time they were captured (chicks with 12 days old), we also did not deploy geolocators on females. Thus, we only present results relative to males here.

We had a substantial difference in the spread of dates between the two years: egg laying April date 2013: mean 38.63, s.d. ± 4.81; egg laying April date 2014: mean 36.41, s.d. ± 8.36. Thus in all analyses that involved more than one year, we centred the dates (expected or manipulated hatching dates) around the mean date instead of using the raw dates as fixed effects to avoid mixing up within year effects caused by our treatments and the natural variation that occurred between years. This procedure was based on all data collected for our population, not only experimental nests. Although some individuals were present in both years of data collection, they were too few (six birds) to allow the use of "individual" as a random effect in a mixed effect model approach. Therefore, if birds were present in the experiment twice, we had to randomly exclude one of the observations from the dataset.

In most cases, we used two separate models: 1) a model including the "expected hatching date" (henceforth "expected hatching date", *i.e.* the egg-laying date + 18 days of hypothetical clutch completion and incubation), which represents the non-manipulated value for seasonal changes and 2) a model including the real hatching date (henceforth "manipulated hatching date"), the actual date of hatching after the manipulations took place. Results arising from these models are complementary and important to be looked at in conjunction to discriminate between the described scenarios.

Ia. Linked (termination of breeding): If the timing of moult and/or departure is causally affected by the termination of breeding, the moult and/or departure dates will differ between treatments when analysed in relation to the expected hatching date. Thus, when data are plotted in relation to the expected hatching date there will be three parallel lines. *i.e.* a significant difference of moult and/or departure date between treatments. In other words, for a given date, the line of the advanced broods it will fall earlier

(below) than the control, and the line of the delayed broods will fall later (above) than the control (Fig. 2a). While, when data are plotted in relation to manipulated hatching date, the points will fall on the same line, because the lines are simply moved along the x-axis (Fig. 2b).

Ib. Linked (other stage): If the variation of moult and/or departure is correlated to the natural variation of another stage (for example, the egg-laying date of the female) without a causal relation to the termination of breeding, the moult and departure dates will not differ between treatments when analysed in relation to the expected hatching date. Thus, when plotted in relation to the expected hatching date, the three lines will fall on top of each other, with no significant treatment effect. Meaning that, for a given hatch date, there will be no difference in timing of moult or departure between advanced, control or delayed (Fig. 2c). But when plotted against the manipulated hatching date, the treatments will be seen as separate parallel lines simply because the lines move along the x-axis (Fig. 2d).

II. "Independence": Finally, it is also possible to have a complete lack of effect of the previous stages on the timing of moult and/or migration, meaning that the timing of moult and departure is solely regulated by photoperiod. This will be seen as the same onset of moult and/or departure, for all individuals, regardless of when they bred (Fig. 2e – f).

b) Timing of breeding, moult and migration

To be sure that there were no differences between the birds due to chance prior to the experimental manipulations, we used multiple linear regressions to test whether the pre-manipulation males` arrival and females' egg-laying dates differed in relation to treatment.

We then tested the effects of our manipulations on hatching dates, prolactin concentration, autumn migration and wintering longitude also using multiple linear regressions. Except for the hatching dates all tests were carried out with the two above-mentioned models (in relation to the centred expected or manipulated hatching dates). Because date effects are not necessarily linear, in all cases we also tested for the quadratic effect of (expected/manipulated) hatching date. The analysis also included "year" as a covariate if we had two years of data (all traits except prolactin concentration). We also tested in separate

analyses if departure date was explained by the onset of moult or the previous un-manipulated arrival date of the male to the breeding grounds.

Out of 102 males identified in the two years (from 111 experimental nests), we could reliably calculate the individual onset of moult for 80. The missing birds were either not captured on chick day 12 (N = 10) or were not moulting by the time they had to be released (N = 12), as we wanted to guarantee the same chances of return for all experimental birds. Therefore, the comparison between the onset of moult in males from advanced, control and delayed groups could not be done using a simple mean group comparison. Instead, we analysed moult onset using Cox proportional hazard models (Cox 1972), since this model allows inclusion of "censored" data and was much more reliable to compare the onset of moult in our different groups. Moult onset was, therefore, our "time to event" variable and the last day of observation of not moulting birds was included in the analysis as "censored". As in previous cases, to test the effect of treatment we used two separate models correcting for either the (linear/quadratic) centred expected or manipulated hatching date, with "year" as a covariate.

The amount of moult-breeding overlap is a consequence of how moult is scheduled in relation to the termination of breeding. An analysis using this trait has the disadvantage to only allow the inclusion of non-censored data, but, on the other hand, it allows to use multiple regressions instead of hazard models, facilitating the visualisation of the results. We thus analysed the moult-breeding overlap (excluding the non-moulting individual) as a complementary way of assessing the potential effects of our treatments. Moult-breeding overlap was calculated as the difference in days between the day of onset of moult and the day when chicks were/would be 15 days old (the fledging date). Negative values represent an overlap, while positive values indicate an interval between fledge dates and onset of moult. It was tested in a similar way of the previous multiple regressions correcting for either the centred expected or manipulated hatching date as linear and quadratic terms, also including "year" as a covariate.

c) Fitness components

We used generalised linear models in R (logit-link and Binomial error-distribution) to test if the probability of brood failure was explained by treatment and a linear regression to test for the effect of treatment on the number of fledgelings. In both cases, we tested in two separate models the effect of treatment correcting for either the centred expected or manipulated hatching date (both as linear and quadratic terms), also including year and starting number of chicks as a covariate.

For adult and chick body masses we used mixed effect models with the brood the chick was born (ontogenetic effect) and original brood (genetic effect) as the random effects and expected or manipulated hatching date as the fixed effect. Year was also included as fixed effect for adult and chick mass and tarsus length was used as fixed effect as a correction factor for structural size. We also included the three-way interaction between year, treatment and hatching date. Because not all chicks could be assigned to a genetic mother, we could not include all of them in this analysis.

To test for treatment effects on number of visits, we also used mixed effect models and included treatment, year, sex, chick age (9, 10 or 11 days old) and their interactions as fixed effects. Because we had males and females feeding the same nest, we also fitted individual and nest as random effects.

Finally, feather quality (bending/stiffness) was analysed using multiple regression, with treatment and year as explanatory variables.

Control experiment

To test for possible differences caused by making clutches of eggs based on the lay order (first and second eggs make up the advanced group, third and sixth egg the delayed group and fourth and fifth egg that make up the delayed group) we designed a control experiment in which we created clutches containing only eggs from those laying orders, but without manipulating the chick hatching dates. This experiment was conducted in 2013 in a separate breeding population in the forests of Buunderkamp (the Netherlands; 5°45′E, 52°01′N). Triplets of nests (N=8 triplets, 24 nests but one control nest was lost due

to the death/desertion of the female) with same egg-laying dates were used to form a group and one of the treatments was randomly assigned to each of the nests: clutch containing only eggs 1 and 2, clutch containing only eggs 3 and 6, or clutch containing only egg 4 and 5. On the day the females laid the sixth egg, eggs were swapped among the nests and treatments were created.

We used multiple regressions to test for effects of treatment on chick hatching dates and hatching success (with egg-laying or chick hatching dates as covariates) and mixed effect models with brood as random effect to test for effects of treatment on chick mass (with egg-laying or chick hatching dates as covariates both as linear and quadratic terms).

There was no effect of treatment on hatching dates ($F_{2,20} = 0.27$, $p = 0.77$, advanced = 56.75 ±0.66, control = 57.43 ±0.71, delayed = 57.25 ±0.66), egg hatching success ($F_{2,20} = 1.93$, $p = 0.17$, advanced = 5.88 ±0.22, control = 5.57 ±0.24, delayed = 5.25 ±0.22) or chick weight ($F_{2,19.99} = 0.57$, $p = 0.58$, advanced = 13.64 ±0.32, control = 13.69 ±0.35, delayed = 14.09 ±0.33). This indicates that any effect of our manipulations on timing was solely caused by the shifts in timing of egg-hatching and not due to possible differences in egg quality correlated to the egg-laying order

Ethical statement

The Animal Experimental Committee of the Royal Netherlands Academy of Sciences (KNAW) approved all experimental procedures, following the Dutch law regulating animal experimentation (protocols NIOO 13.04 and amendment, NIOO 14.04). Eggs of foster parents were removed from foster nests prior to incubation and discarded (under European law eggs are not considered vertebrates) and foster parents (great and blue tits) had their nests removed after the experiment. In some cases, those birds were re-sighted in the study site starting a new brood. Occasional seventh eggs of pied flycatchers were removed and, if not used to complete clutches, were sampled for DNA to estimate extra-pair paternity in a separate study (Tomotani et al., 2017). Experimental manipulations did not affect the probability of the pied flycatcher chicks to fledge: nine out of 97 experimental nests (over both years) failed at some point of the experiment, which is within the natural range of brood success for our area. Out of those nine nests, three failed immediately after hatching before the adults were caught, five failed

between the first and the second capture and only one failed after the male was taken to the laboratory. The number of fledged chicks was not different between manipulated and un-manipulated nests. When kept in the laboratory, birds initially decreased their body weight in the first three days and then returned to their normal weight, sometimes becoming heavier prior to release. It is not possible to compare the return rates of the manipulated and un-manipulated birds in our population during those two years because the majority of the un-manipulated birds were the late-breeders. In comparison to the average survival across years, the return rate of the manipulated males was lower than the average survival probability for our population (21% versus 47%). However, our recovery success of birds deployed with geolocators in 2013 was similar to that reported for a nearby population (32% versus 34%, Ouwehand & Both, 2018). Therefore, while it is possible that our manipulations have affected the survival of the birds, year-to-year differences in survival may also have played a role (with a naturally lower survival in 2014).

Results

Full statistical tables can be found in the supplements.

Effect of the manipulation

There was no difference in arrival dates or egg-laying dates of birds in different treatments prior to the experiment. Egg hatching dates were 3.5 d earlier in the advanced group and 3.5 d later in the delayed group relative to the control, showing that our experiment had the intended effect ($F_{1,98}$ = 268.01, *p*-value <0.01, Fig. 3a–b). Therefore, we are confident that our experimental manipulation of hatching dates was successful.

Timing of moult

There was no effect of treatment on the onset of moult, independently of using the expected or the manipulated hatching dates (treatment term: X^2 = 2.25, p=0.32; Fig. 3c, d). There was also no linear or non-linear effect of (expected or manipulated) hatching date on the onset of moult. Those results are in

accordance with our **scenario II: Independence** and suggest that all birds had a similar moult onset that did not depend on termination of breeding (Fig. 3c, d). In addition, there was a significant year effect (average onset of moult in June 16th in 2013 and June 11th in 2014; $X^2 = 16.13$, $p<0.01$).

In line with this lack of adjustment of moult in relation to breeding, birds in the delayed treatment had a larger amount of moult-breeding overlap than advanced and controls when controlling for the expected hatching date ($F_{2,69} = 4.00$, p-value = 0.02, advanced = 4.38 ±1.61, control = 3.14 ±1.61, delayed = -0.71 ±1.45, Fig. 3e). There was also a significant quadratic effect of the expected hatching date ($F_{2,69} = 7.88$, p-value = 0.01, estimate = -0.07 ±0.03, Fig. 3e); i.e. the weak effect of hatching date was only found in very late birds. However, when we compared this quadratic trend with two linear regressions (*post hoc* "broken-stick" analysis), we found that two regressions had a better fit than the quadratic term (adjusted R^2 for quadratic term = 0.47, adjusted R^2 = 0.5 for two regressions with a break point at centred expected hatching date of 5). Dividing the dataset before and after centred day 5 and testing the two regression separately we found that the first regression was significant ($F_{1,60} = 29.8$, $p<0.01$, slope = -1.04 ±0.19, Fig 3e), but the second one, that included the late breeders, with a much lower sample size, was borderline non-significant ($F_{1,5} = 0.8$, p-value =0.05, Fig. 3e). Finally, the relationship between moult-breeding overlap and manipulated hatching date is significant and very close to 1 ($F_{1,72} = 50.4$, $p<0.01$, slope = -1.06 ±0.15, Fig. 3f), so for each day that hatching is delayed, overlap increases by one day. This supports the moult onset analysis and suggests that onset of moult is similar for all birds, being independent from the termination of breeding.

Prolactin concentration

The prolactin concentration measured in males when chicks were 12 days old was significantly higher in advanced birds and similar in control and delayed birds in the model controlling for the expected hatching date (treatment term: $F_{2,31} = 4.43$, p-value = 0.02, advanced = 7.94 ±2.47, control = 7.15 ±2.47, delayed = 7.16 ±2.41; expected hatching date term: $F_{1,31} = 5.42$, p-value = 0.03, slope = -0.15 ±0.07, Fig. 4a), with the treatment effect disappearing in the model that uses the manipulated hatching date (manipulated hatching date term: $F_{1,33} = 11.62$, p-value = 0.01, slope = -0.12 ±0.04, Fig. 4b). This is in

agreement with our **scenario Ia. Linked (termination of breeding)** and suggests a causal effect of hatching date on prolactin concentration. Thus, advanced birds had the same prolactin levels as individuals that had a naturally earlier breeding time and were "moved" to an earlier point in the curve of natural seasonal decline in prolactin. This also means that chick age did not affect the prolactin levels.

Timing of migration and wintering longitude

Males departed from the breeding grounds between 24 July and 18 August, which was on average 50.5 days after fledging their chicks, and 33.5 days after release from the lab. We retrieved 32% (7 out of 22) of the advanced males with geolocators, 36% (8 out of 22) of the controls and 17% of the delayed (4 out of 24) (but these differences are not significant, $X^2 = 2.58$, p-value = 0.28).

The date of departure from the Netherlands to Africa was positively correlated with the expected hatching date ($F_{1,17} = 43.16$, p-value = 0.01, slope = 1.19 ±0.18, Fig. 3g), with no indication of an additive treatment effect (Fig. 3g), supporting the **scenario Ib. Linked (other stage)**. Thus, birds that naturally bred earlier also departed earlier. However, according to our prediction (Fig. 2), we would also expect to find a significant treatment effect, which was not the case ($F_{2,15} = 1.5$, p=0.27, Fig. 3h). One possibility for this result is that we have a clear segregation of treatments across the manipulated egg-hatching date axis since this is the essence of our manipulations (*i.e.*, early-hatched were primarily advanced and late-hatched primarily delayed). This makes the testing of a treatment effect difficult as the model will fit a line through three discrete groups of points which are at different positions on the x-axis. To correct for this, as a *post hoc* test, we performed a second analysis constraining the slope of each treatment to the same general slope of the expected hatching date term (obtained in the previous analysis with treatment corrected for expected hatching date = 1.19). With this constrained slope, this second analysis pointed to a significant treatment effect ($F_{1,16} = 3.95$, p-value = 0.04, estimate in the presence of the slope: advanced = 138.60 ±1.70, control = 134.94 ±1.59, delayed = 130.78 ±2.24), which thus does support the **scenario Ib. Linked (other stage)**.

Departure date was not correlated with the individual moult onset ($F_{1,15}$ = 2.64, *p*-value = 0.12, slope = 0.39 ±0.24, excluding non-moulting birds) nor to the arrival date to the breeding grounds earlier in the same breeding season ($F_{1,12}$ = 0.28 , *p*-value = 0.61, slope = 0.27 ±0.51).

Wintering longitude was significantly affected by treatment ($F_{2,16}$ = 3.96, *p*-value = 0.04, advanced = -7.40 ±0.45, control = -7.00 ±0.42, delayed = -9.01 ±0.59, Fig. 5), with delayed birds wintering ca. 200 km more westerly than advanced and control birds. We did not find year effects on wintering longitude, however, we found a curious additional effect of natural hatching date, but in the opposite direction of our treatments with naturally early birds wintering more westerly than late ones (natural hatching date: $F_{1,15}$ = 5.13, *p*-value= 0.04, slope= 0.11 ±0.05).

Fitness components

When breeding success was analysed, there was no significant treatment effect on the probability of brood failure and neither on the number of 12 day-old chicks. The final number of chicks was obviously dependent on number of chicks hatched (5 or 6), which was not related to treatment ($F_{1,100}$ = 11.50, *p*-value < 0.01, slope= 0.82 ±0.24).

There were no treatment effects on the fitness components measured for the adult birds. We found a significant three-way interaction between treatment, sex and year on the weight of adults, measured when their chicks reached 12 days old ($F_{3,80.42}$ = 3.61, *p*-value = 0.02). However, when the dataset was divided and 2013 and 2014 analysed separately, there was no significant treatment difference. The small sample size may have produced a misleading pattern when a three-way interaction was fitted, therefore, for the present work we should assume that there was no relation between treatment and adult weight. The only significant difference was between sexes in 2013, with males being lighter than females ($F_{1,44.83}$ = 9.21, *p*-value < 0.01). There was also no difference between treatments in the quality of the male feathers (feather bending/stiffness), produced after our manipulations and collected in the following year. Finally, there was no effect of treatment on the number of visits to the nest per day. From all traits tested, only sex had a significant effect, with females feeding significantly more than males ($F_{1,71.26}$ =

13.62, *p*-value < 0.01). Effects of individual birds (as random effects) were also significant (P<0.01) and explained 73% of the variance, since, as expected, some individuals visit the nest much more often than others.

There were also no treatment effects on the fitness components measured for the chicks. Chick weight was not causally affected by treatment as it is evident in the non-significant treatment effect in the analysis including the egg-laying date. There was a three-way interaction between treatment, hatching date and year ($F_{2,151.41}$ = 6.08, *p*-value < 0.01) but, again, when the dataset was divided and 2013 analysed separated from 2014, both treatment and hatching date-laying date effect disappeared. This means that there was no effect of treatment, laying date or hatching date on chick weight.

Carry-over effects (reversible state effects)

Treatments did not differ in the probability of finding the manipulated birds the next year (X^2 = 0.83, *p*-value = 0.66) or any of following years (up to 2016, X^2 = 1.04, *p*-value = 0.59). The breeding date of males and females in the following year was also not affected by treatment ($F_{1,26}$ = 0.39, *p*-value = 0.68), only by their un-manipulated laying date in the previous year (estimate = 0.58 ±0.27, $F_{1,40.98}$ = 4.56, *p*-value = 0.04) and the year in which the experiment was conducted (2013 = 11.66 ±10.15, 2014 = 17.13 ±9.61, $F_{1,39.72}$ = 8.73, *p*-value = 0.02).

Discussion

Our results show that in male pied flycatchers, the timing of moult is independent of the termination of breeding (**scenario II. Independence**). Moult, however, is also not completely dependent on photoperiod since moult onset varies from year to year (Fig. 6). Alternatively, moult could dependent on a so-called “remote timer” (Helm and Gwinner, 2005). The timing of autumn migration, on the other hand, depends on a previous stage, but not on the termination of breeding (**scenario Ib. Linked, other stage**). Therefore, male pied flycatchers do not compensate for the earlier or later hatching of their chicks by changing the onset of their moult or their departure date. Instead, if hatching date is shifted to an

earlier or later date, it affects the degree of overlap between breeding and moult. Although we did not detect short-term effects on fitness caused by our treatments, delaying the chick hatching dates caused adult males to winter at a different location (Fig. 5), suggesting carry-over effects of moult-breeding overlap on wintering behaviour. However, these effects disappear in the following year consistent with a "reversible state effect" (Senner et al., 2015).

While our results suggest that the timing of moult is relatively inflexible for most individuals, other studies have suggested that timing of moult in males is related to breeding. For example, (Dawson, 2006) showed in starlings a relation between moult onset and gonadal regression. Because we did not collect data on testicular regression, it is not possible to infer whether moult in our birds was or was not correlated with gonadal regression. This aspect must remain speculative, but we do not discard it as a possibility. The interpretation of whether or not breeding affects moult thus may depend on how "termination of breeding" is defined. For our purposes in the present study, when assessing overlap of annual-cycle stages it seems to be more meaningful to assume the end of chick care as the termination of breeding, especially in bird species where both parents contribute to chick care such as pied flycatchers.

Hemborg (1998) also conducted an egg manipulation experiment with pied flycatchers and showed that advanced and delayed males did not differ in their post-breeding moult stage, which is the opposite of what we found. Because our experimental design is not directly comparable to his (*i.e.*, his design depended on broods that lay eggs a number of days apart, while the present experiment uses broods with same laying dates), it is difficult to determine what exactly caused these differences. Pied flycatchers from Sweden breed later than birds from the Netherlands and are supposedly more time constrained to moult (Hemborg had up to 80% of the males and 50% of the females moulting at chick day 13, while we had at most 24% and 6%, respectively, at chick day 12); thus, they may require some different strategies to be able to migrate in time (Hemborg et al., 1998; Hemborg et al., 2001). An inflexible timing of post-breeding moult was also found in other long-distance migrants such as the bluethroat, *Luscinia svecica* (Lindström et al., 1994). It is, thus, possible that flexibility in the timing of post-

breeding moult varies among species and populations (Helm and Gwinner, 2006; Jenni and Winkler, 1994; Newton, 2009).

In our experiment, an independent timing of moult is supported by the prolactin results. There is good evidence for the role of prolactin in the regulation of moult (Dawson, 2006; Dawson and Sharp, 2010; Payne et al., 1972; Williams, 2012). For instance, administration of prolactin leads to moult inhibition in some species (Payne et al., 1972), while a decline in prolactin (but not the hormone level *per se*) could act as the moult trigger (Dawson, 2006). We observed higher levels of prolactin in advanced birds in comparison to control and delayed animals (Fig. 4), together with a smaller amount of moult-breeding overlap in birds from the advanced group (Fig. 3). We would expect equally high levels of prolactin in the same chick age if the moult control depended on the termination of breeding. Thus, the prolactin results also support the **scenario II. Independence** for moult onset.

The lack of effect of the manipulations on the timing of moult, and moult occurring at a certain calendar day for most birds, suggests that moult is either independent of hatching or that the decision to start moulting is made at an earlier, un-manipulated, stage in the season than hatching date. While our experiment is not able to test for causality of other stages, it is possible to test for these two possibilities using the natural variation in moult onset. For a few individuals, we had data on moult and laying dates in both 2013 and 2014. We used these data to compare for each individual how the difference in laying dates between 2013-2014 was related to the 2013-2014 difference in moult onsets. These results are present in Figure 6: If moult onset is related to a previous stage, such as laying date, points would fall on the hypothetical **line a**, while a complete independence would make the points fall on the hypothetical **line b**. We found that moult onset did not depend on a previous stage (slope was not significantly different from 0, $F_{1,8} = 2.92$, $p = 0.13$; but was from 1, $F_{1,8} = 21.2$, $p<0.01$), not falling on **line a**. But it is also interesting to notice that it also did not fall on **line b**, so it does not happen exactly at the same day in different years (intercept was significantly different from 0, estimate = -7.38, $p<0.01$). These results suggest that timing of moult is not solely regulated by photoperiod and may also be related to an environmental variable that that varies from year-to-year. Alternatively, flycatchers could use of a "remote timer", and the timing of moult is set during spring migration. Because there is year-to-year

variation in photoperiodic exposure due to a year-to-year variation in timing of migration there would also be variation in timing of moult (Helm and Gwinner, 2005; Sharp, 2005).

In contrast to the breeding/moult transition, information on the transition to the migratory phenotype is more sparse in the literature. Some studies have demonstrated a possible hormonal control mediated by testosterone and corticosterone in the transition between spring migration and breeding (Ramenofsky and Wingfield, 2006; Tonra et al., 2013) and also in stopover decisions (Eikenaar et al., 2017). Autumn migration, however, may still have a different control (Agatsuma and Ramenofsky, 2006; O'reilly and Wingfield, 1995). Our data suggest that departure time from the breeding grounds is related to the onset, but not the termination of breeding (Fig 3). While we are unable to assume causal relations due to the lack of experimental manipulations of these other stages, it is still interesting to speculate. We found a positive correlation between the arrival of the males and the egg-laying date of their females (used as a proxy for the expected hatching date, prior to experimental manipulation, estimate: 0.52 ±0.16, $F_{1,69}$ = 10.46, p<0.01) and also between the egg-laying dates of the females and the males' departure back to Africa (estimate: 8.09 ±2.47, $F_{1,14}$ = 10.71, p<0.01, see also Ouwehand and Both (2017) for a similar trend). This suggests that male departure date is set relatively early in the season and may even be related to timing of spring arrival, although our data do not show a significant correlation between male spring arrival and autumn departure dates (but our sample sizes are relatively small). This suggests that male pied flycatchers cannot or will not delay their departure to accommodate a late chick hatching and neither delay their moult. A delay in chick hatching or moult would increase the time pressure for chick rearing and growth and adult feather replacement, with potential fitness costs (Dawson, 2004; Tomotani and Muijres, 2019; Tomotani et al., 2016; Vágási et al., 2012).

It is striking that flycatchers depart soon after the breeding season and that males relatively frequently bear the costly overlap between chick feeding and moult, suggesting a great benefit to depart early. Interestingly, the ongoing advance in mean laying date in our breeding population since 1980 (Tomotani et al., 2018a; Visser and Gienapp, 2019; Visser et al., 2015) could have led to some relaxation in moult-breeding overlap, because moult was found to be unrelated to laying date within years. However, years did differ in start of moult, being earlier in the warmer 2014 than the colder 2013, which suggest that

this annual-cycle stage may also vary over the years. Indeed, in the last 10 years, male moult onset have advanced faster than breeding (Tomotani et al., 2018a). Autumn departure has also been shown to advance in single brooded-species migrating through Switzerland (Jenni and Kery, 2003), although not clearly in citizen science observations in Britain (Newson et al., 2016). It is thus not clear yet how a change in timing of one annual-cycle stage over time impacts on later stages, but for pied flycatchers, it seemed that any delays during autumn migration are compensated during winter (Ouwehand and Both, 2017).

It is interesting to point that we did not find short-term fitness costs of moult breeding overlap as there was no effect of treatment on any component measured. Williams (2012) suggested that there is a lack of empirical evidence in the literature supporting the energetic costs of overlapping moult and breeding (but see Tomotani and Muijres, 2019; Tomotani et al., 2018b). However, such costs could appear at a later stage in the annual-cycle (Williams, 2012). Our data support this idea, since birds in different treatments also differed in their wintering location, with delayed birds wintering further west than advanced and control birds (Fig. 5). Interestingly, the differences in wintering longitude that we observed resemble the population difference of pied flycatchers seen across different European breeding populations (Ouwehand et al., 2016), in which naturally late breeding populations wintered further west than naturally earlier populations. A similar effect was, however, not observed in birds that naturally breed earlier or later neither in our population nor in another Dutch flycatcher population deployed with geolocators (Ouwehand and Both, 2017). These observations support a causal relation between moult-breeding overlap and wintering behaviour. We can speculate that such a pattern appears due to: **(i)** a change in body condition of birds in the delayed treatment that made individuals less competitive to secure a winter territory and/or made them less loyal to their former wintering site – although this could also lead to a later autumn departure, for which we found no evidence; or **(ii)** the birds that we recovered were the ones that changed behaviour and wintered in a different location, while the others did not return to the breeding grounds and thus are not represented in our dataset. In any case, we suggest that delaying breeding may have carry-over effects on the wintering distribution of pied flycatchers.

Finally, the lack of causal relationship between the termination of breeding, moult and departure means that these three life history stages may be differently affected by climate change. When climate change advances timing of breeding, but timing of moult does not shift at the same rate, this would either increase the time available between breeding and moult (thus reducing the probability of moult breeding overlap) or increase the amount of overlap. However, this overlap will increase when moult advances faster than egg-laying dates (Dawson, 1998; Tomotani et al., 2018a; Visser et al., 2009). Even if some time is gained between moult and breeding, there will be time loss in another portion of the cycle, as was already seen in the timing between arrival and breeding in the Hoge Veluwe population of pied flycatchers (Both and Visser, 2001; Tomotani et al., 2018a). In summary, our results suggest that while the advancement of breeding caused by climate change will increase the time pressure for arrival dates, it may relax the pressure for summer moult and fall migration, and this way may lead to a mismatch of annual-cycle stages.

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Competing interests

No competing interests declared

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Data Availability

Data supporting this manuscript will be available at the Dryad Digital Repository upon publication.

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Figures

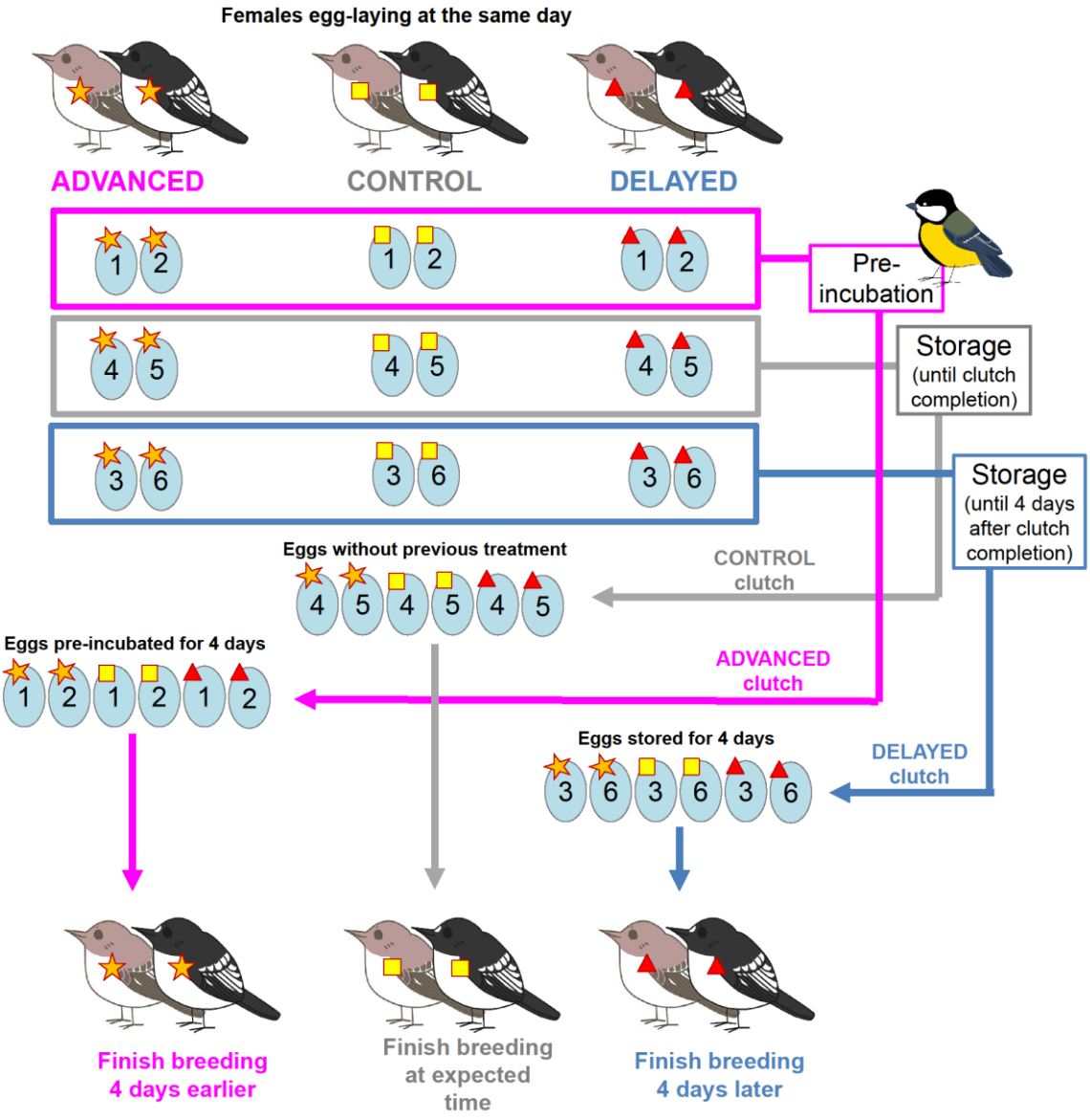

Figure 1: Illustration summarising the experimental design. A group is formed when we find three females laying their first egg at the same day, nests are randomly assigned to a treatment at this moment. **Advanced:** receive eggs number 1 and 2 from all nests. As soon as egg number 2 is laid, all 1 and 2 eggs are immediately placed under an already incubating great tit, causing development to start 4 days before the natural date. Eggs are given to the advanced nest when the flycatcher female starts to incubate. Eggs hatch 4 days prior to the expected hatching date, resulting in an earlier termination of breeding. **Control:** receive eggs number 4 and 5 from all nests. Those eggs are stored and given to the control nest upon clutch completion. Eggs hatch at the expected date and result in a termination of breeding at

the expected time. **Delayed:** receive eggs number 3 and 6 from all nests. Those eggs are stored and only returned 4 days after clutch completion. Eggs hatch 4 days after the expected hatching date, resulting in a later termination of breeding. This procedure is repeated throughout the whole season. Numbers on eggs represent egg-lay order, different symbols (stars, squares and triangles) represent distinct families (genetic parents) from which the original clutch came.

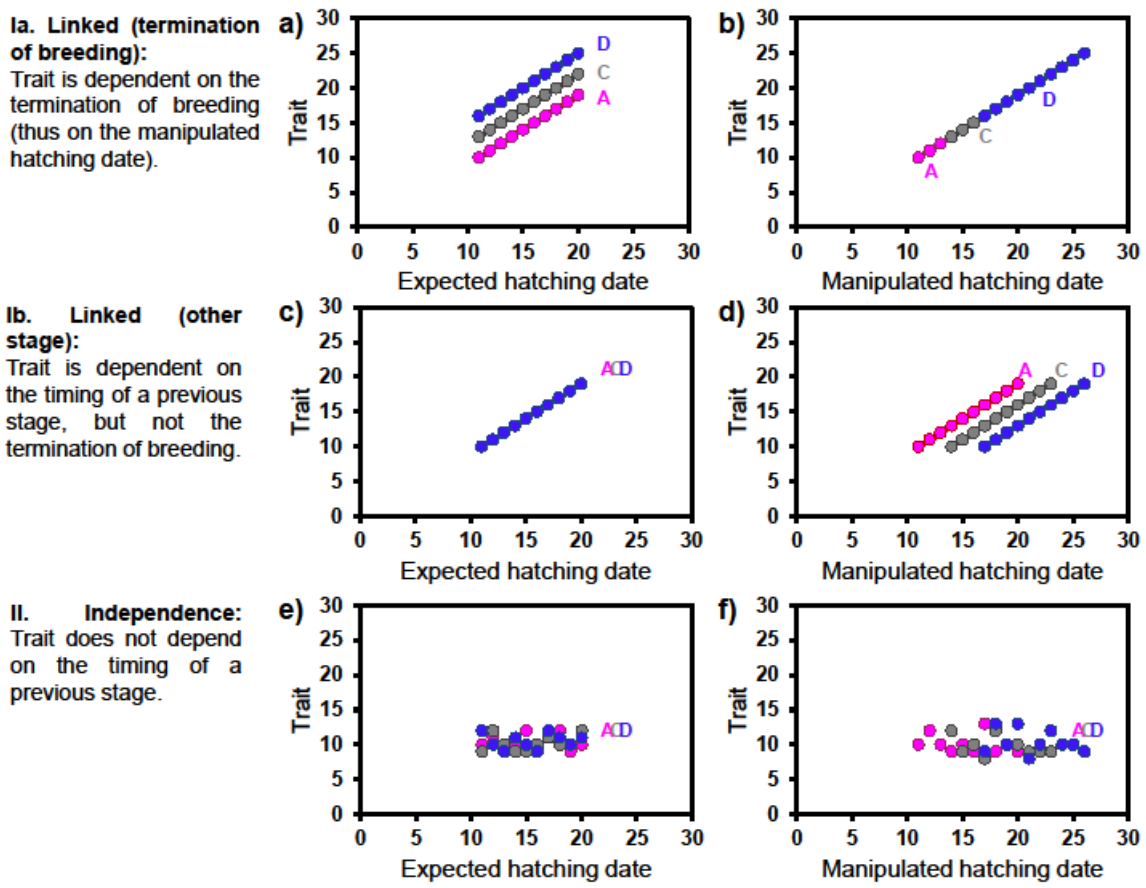

Figure 2: Possible outcomes of the experiment related to the different scenarios. **a)** If the trait depends on the termination of breeding, for a given expected hatching date, the trait value will be lower (earlier) for advanced and larger (later) for the delayed in relation to the control and will vary depending on the expected hatching date. **c)** If the trait depends on the timing of another non-manipulated stage for a given expected hatching date, the trait value will not differ between advanced, control or delayed, but will vary depending on the expected hatching date. **e)** If the trait does not depend on the timing of a previous stage, the trait value will be the same regardless of treatment or expected hatching date. **b,d,f)** Complementary plots based on the manipulated hatching date (advanced = expected hatching date – 4 days; control = expected hatching date + 0 days; delayed = expected hatching date + 4 days). Lines are simply moved along the x-axis depending on the treatment. Colours represent the three treatments (pink: advanced, grey: control, blue: delayed). The difference between manipulated hatching dates is always 4 days.

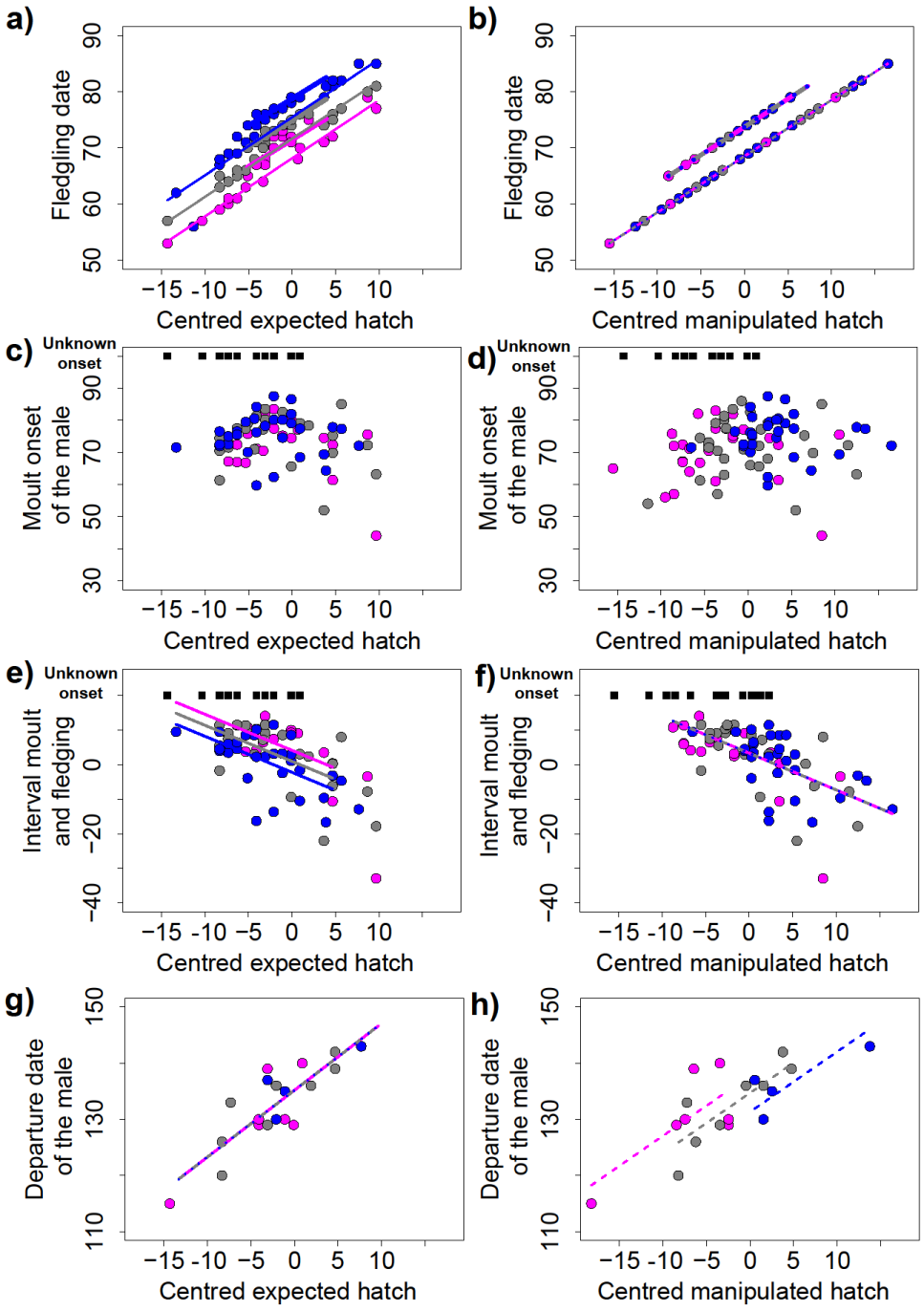

Figure 3: Calculated fledgling date (date when chicks were 15 days old) in April days (**a, b**), moult onset of the male in April days (**c, d**), moult-breeding overlap of the male (difference between onset of moult and fledgling date) (**e, f**) and departure date of the male in April days (**g, h**) in relation to the centred expected hatching date, using laying date as a proxy (**a, c, e, g**) and the centred manipulated hatching date, the actual hatching date (**b, d, f, h**). Colours represent each of the treatments: pink = advanced (n= 37), grey = control (n=36), blue = delayed (n=38). Black squares represent males that were not moulting when they were checked for the last time. Details on plotted model predictions: In

(a) Model predictions are plotted for each treatment (pink = advanced, grey = control, blue = delayed) and year (bold = 2013, n=, non-bold=2014). In **(b)** Bold = 2013, non-bold=2014. In **(e)** model predictions come from the broken-stick analysis. In **(b**, **f, g)** only the date as a linear term is significant, treatment lines are overlapping. In **(h)** Treatments are only significant when slope is constrained. Moult onset: **scenario II. Independence**; Departure date: **scenario Ib. Linked (other stage).**

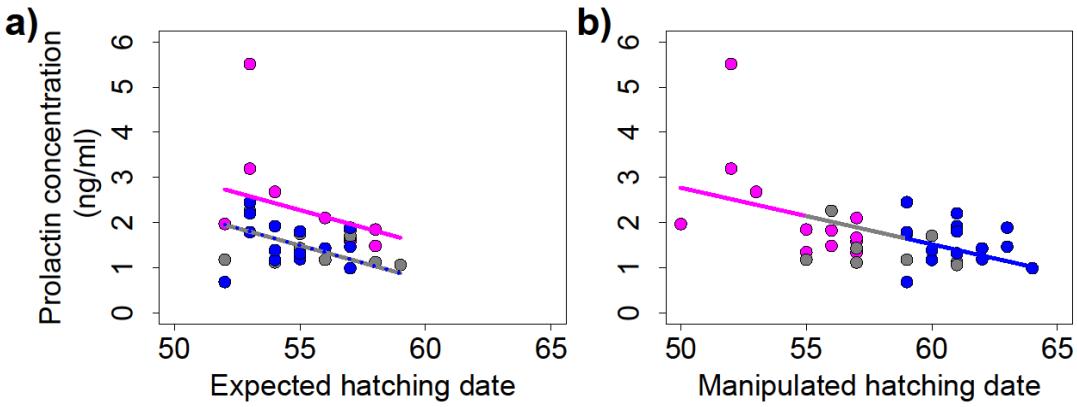

Figure 4: Prolactin concentration (ng/ml) of males measured when their chicks were 12 days old in relation to **a)** the egg-laying date of their females or **b)** the hatch date of their chicks in April days. Different colours represent each of the treatments: pink = Advanced (n=12), grey = Control (n=9), blue = Delayed (n=14). The lines represent the predictions for each treatment in their respective colour. Grey and blue lines are overlapping in figure 4a, all lines are overlapping in figure 4b. Prolactin: **scenario Ia. Linked (termination of breeding).**

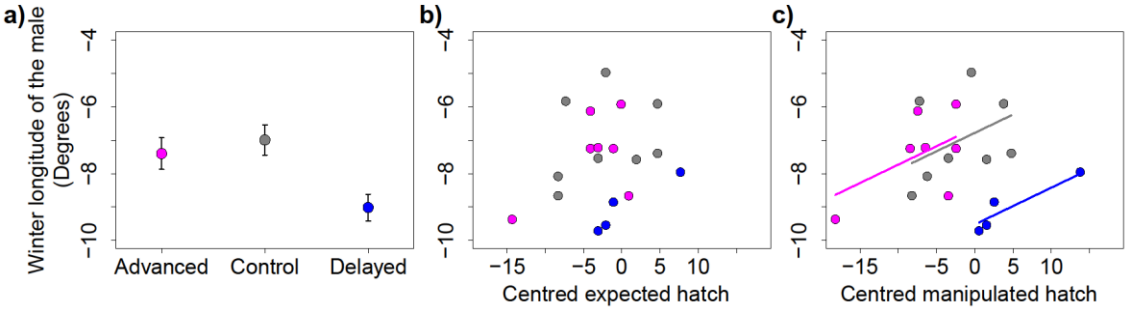

Figure 5: Males' wintering location (in degrees) as **a)** mean ±s.e.m. for each treatment or individual values in relation to the centred **b)** expected or **c)** manipulated hatching date. Pink = advanced (n=7), grey = control (n=8), blue = delayed (n=4).

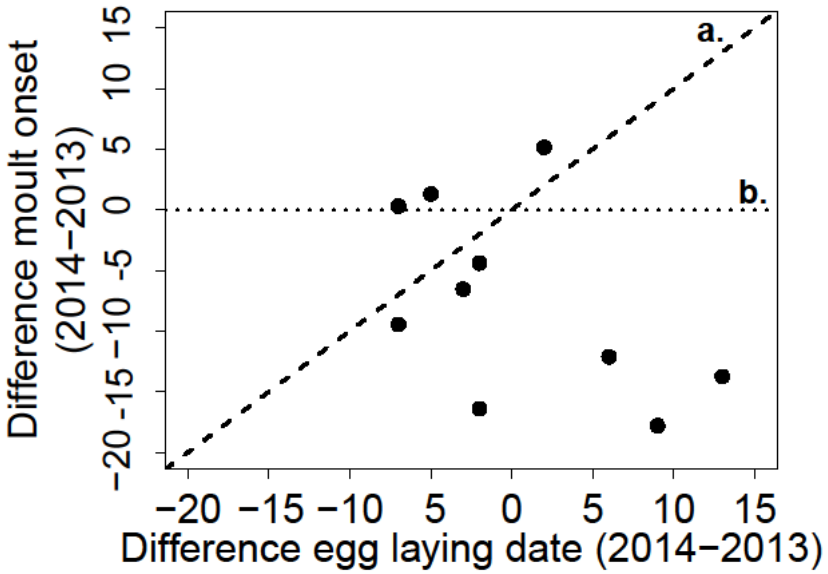

Figure 6. Relationship between the difference in moult onset in 2013 and 2014 and the difference in egg laying date in 2013 and 2014 in the same individuals (n=17). **Line a**. represents the situation in which moult depends on a previous stage. **Line b**. indicates that moult is independent of a previous stage and if the intercept is 0 that it happens every year on a certain day.

Table S1: Timing components. Model results for the multiple regression analyses explaining the timing components before (arrival and lay dates) and after the manipulations (hatch date, prolactin, moult-breeding overlap, departure date and wintering location) and moult onset analysed with proportional hazard models. Analyses were done either with the expected hatching date (using laying date + 18 days of expected clutch completion and incubation duration as a proxy) or manipulated hatching date (actual hatch date). Statistics are given for each term at the point of the exclusion of the term from the model. Estimates and standard errors are presented only for significant terms or main terms in the presence of significant interactions. In the case of significant interactions with continuous terms and factors, we don't provide statistics for the continuous main effects.

1) Arrival date (NL - prior to treatment)	**Estimate**	**s.e.**	**ndf**	**ddf**	**R^2 (adjusted)**	***F*-test**	***p*-value**
Treatment : Year			2	66	0.08	2.52	0.09
Year			1	68	0.04	0.43	0.51
Treatment			2	69	0.05	2.87	0.06
2) Egg-laying date (prior to treatment)	**Estimate**	**s.e.**	**ndf**	**ddf**	**R^2 (adjusted)**	***F*-test**	***p*-value**
Treatment : Year			2	97	0.05	0.05	0.95
Treatment			2	99	0.07	0.02	0.98
Year			1	101	0.98	10.30	**<0.01***
Year (2013)	37.49	0.74					
Year (2014)	34.20	0.71					
3) Egg-laying date (prior to treatment)	**Estimate**	**s.e.**	**ndf**	**ddf**	**R^2 (adjusted)**	***F*-test**	***p*-value**
Arrival date : Year			1	68	0.19	0.16	0.69
Arrival date (prior to treatmet)			1	69	0.20	10.46	**<0.01***
Year			1	69	0.20	8.58	**<0.01***
Arrival date (prior to treatmet)	0.52	0.16					
Year (2013)	28.31	2.94					
Year (2014)	24.74	2.85					
4) Hatch date (manipulated)	**Estimate**	**s.e.**	**ndf**	**ddf**	**R^2 (adjusted)**	***F*-test**	***p*-value**
Treatment : Centered expected hatch			2	96	0.96	1.85	0.16
Treatment			2	98	1.00	268.01	**<0.01***
Centered lay date			1	98	1.00	1665.07	**<0.01***
Year			1	98	1.00	171.40	**<0.01***
Treatment (Advanced)	56.56	0.27					
Treatment (Control)	60.04	0.27					
Treatment (Delayed)	63.89	0.26					
Centered lay date	1.03	0.03					
Year (2014)	-3.43	0.26					

5a) Moult onset - males (expected hatch)	**Coefficient**		**n**	**Events**	**df**	**χ^2**	***p*-value**
Treatment : Centered expected hatch^2			93	73	2	0.71	0.70
Treatment : Centered expected hatch			93	73	2	1.81	0.40
Centered expected hatch^2			93	73	1	2.99	0.08
Centered expected hatch			93	73	1	0.04	0.85
Treatment			93	73	2	2.25	0.32
Year			93	73	1	16.13	**<0.01***
Year (2014)	1.01						

5b) Moult onset - males only (manipulated hatch)	**Coefficient**		**n**	**Events**	**df**	**χ^2**	***p*-value**
Treatment : Centered manipulated hatch^2			93	73	2	0.86	0.65
Treatment : Centered manipulated hatch			93	73	2	1.10	0.58
Centered manipulated hatch^2			93	73	1	0.91	0.34
Centered manipulated hatch			93	73	1	0.11	0.74
Treatment			93	73	2	2.25	0.32
Year			93	73	1	16.13	**<0.01***
Year (2014)	1.01						

6a) Moult-breeding overlap (expected hatch)	**Estimate**	**s.e.**	**ndf**	**ddf**	**R^2 (adjusted)**	***F*-test**	***p*-value**
Treatment : Centered expected hatch^2			2	62	0.47	1.33	0.27
Treatment : Centered expected hatch			2	63	0.47	0.08	0.92
Treatment : Year			2	65	0.48	2.11	0.13
Centered expected hatch^2			1	69	0.49	7.88	**0.01***
Centered expected hatch			1	69	0.49	64.64	**<0.01***
Treatment (Advanced)			2	69	0.49	4.00	**0.02***
Year			1	68	0.46	0.15	0.70
Centered expected hatch^2	-0.07	0.03					
Centered expected hatch	-1.28	0.16					
Treatment (Advanced)	4.38	1.61					
Treatment (Control)	3.14	1.61					
Treatment (Delayed)	-0.71	1.45					

6b) Moult-breeding overlap (manipulated hatch)	**Estimate**	**s.e.**	**ndf**	**ddf**	**R^2 (adjusted)**	***F*-test**	***p*-value**
Treatment : Centered manipulated hatch^2			2	62	0.40	0.15	0.86
Treatment : Centered manipulated hatch			2	64	0.41	0.80	0.46
Treatment : Year			2	66	0.42	2.82	0.07
Centered manipulated hatch^2			1	68	0.39	0.03	0.86
Centered manipulated hatch			1	72	0.40	50.04	**<0.01***
Treatment			2	70	0.40	1.08	0.35
Year			1	69	0.39	0.02	0.89
Centered manipulated hatch	-1.06	0.15					

7a) Prolactin concentration (expected hatch)	**Estimate**	**s.e.**	**ndf**	**ddf**	**R^2 (adjusted)**	***F*-test**	***p*-value**
Treatment : expected hatch^2			2	26	0.21	0.26	0.77
Treatment : expected hatch			2	29	0.27	1.98	0.16
Treatment			2	31	0.86	4.43	**0.02***
expected hatch^2			1	28	0.25	0.04	0.84
expected hatch			1	31	0.86	5.42	**0.03***
Treatment (Advanced)	7.94	2.47	2	31	0.86	4.43	**0.02***
Treatment (Control)	7.15	2.47					
Treatment (Delayed)	7.16	2.41					
expected hatch^2							
expected hatch	-0.15	0.07					

7b) Prolactin concentration (manipulated hatch)	**Estimate**	**s.e.**	**ndf**	**ddf**	**R^2 (adjusted)**	***F*-test**	***p*-value**
Treatment : Manipulated hatch^2			2	26	0.27	0.07	0.93
Treatment : Manipulated hatch			2	29	0.29	1.79	0.18
Treatment			2	31	0.25	1.34	0.28
Manipulated hatch^2			1	28	0.31	2.06	0.16
Manipulated hatch			1	33	0.24	11.62	**<0.01***
Manipulated hatch	-0.12	0.04					

8a) Departure date to Africa (expected hatch)	**Estimate**	**s.e.**	**ndf**	**ddf**	**R^2 (adjusted)**	***F*-test**	***p*-value**
Treatment : Centered expected hatch			2	12	0.60	0.17	0.84
Treatment			2	14	0.65	0.17	0.85
Centered expected hatch	1.19	0.18	1	17	0.70	43.16	**<0.01***
Year			1	16	0.68	0.12	0.73

8b) Departure date to Africa (manipulated hatch)	**Estimate**	**s.e.**	**ndf**	**ddf**	**R^2 (adjusted)**	***F*-test**	***p*-value**
Treatment : Centered manipulated hatch			2	12	0.54	0.25	0.78
Treatment			2	15	0.60	1.50	0.26
Centered manipulated hatch	0.84	0.16	1	17	0.58	25.81	**<0.01***
Year			1	14	0.59	0.45	0.52

8c) Departure date fixed slope (Hatching date)	**Estimate**	**s.e.**	**ndf**	**ddf**	**R^2 (adjusted)**	***F*-test**	***p*-value**
Treatment: Advanced	138.61	1.70	2	16	1.00	3.95	**0.04***
Treatment: Control	134.93	1.59					
Treatment: Delayed	130.78	2.24					
Year			1	15	0.66	0.36	0.56

9) Departure date with moult	**Estimate**	**s.e.**	**ndf**	**ddf**	**R^2 (adjusted)**	***F*-test**	***p*-value**
Treatment : Centred expected hatch			2	8	0.48	4.27	0.05
Treatment			2	10	0.40	0.83	0.47
Centred expected hatch	8.09	2.47	1	14	0.39	10.71	**<0.01***
Moult onset			1	13	0.45	1.61	0.13
Year			1	12	0.42	0.26	0.62

10) Departure date with arrival	**Estimate**	**s.e.**	**ndf**	**ddf**	**R^2 (adjusted)**	***F*-test**	***p*-value**
Arrival : Year			1	9	0.02	2.11	0.18
Year			1	10	-0.09	0.28	0.61
Arrival			1	11	-0.02	0.78	0.40

11a) Wintering longitude (expected hatch)	**Estimate**	**s.e.**	**ndf**	**ddf**	**R^2 (adjusted)**	***F*-test**	***p*-value**
Treatment : Centered expected hatch			2	12	0.27	0.40	0.68
Treatment (Advanced)			2	16	0.98	3.96	**0.04***
Centered expected hatch			1	15	0.36	3.92	0.07
Year			1	14	0.33	0.29	0.60
Treatment (Advanced)	-7.40	0.45					
Treatment (Control)	-6.99	0.42					
Treatment (Delayed)	-9.02	0.59					
Centered expected hatch							

11b) Wintering longitude (manipulated hatch)	**Estimate**	**s.e.**	**ndf**	**ddf**	**R^2 (adjusted)**	***F*-test**	***p*-value**
Treatment : Centered manipulated hatch			2	12	0.29	0.20	0.82
Treatment (Advanced)			2	15	0.40	7.51	**0.01***
Centered manipulated hatch			1	15	0.40	5.13	**0.04***
Year			1	14	0.38	0.36	0.56
Treatment (Advanced)	-6.63	0.53					
Treatment (Control)	-6.78	0.39					
Treatment (Delayed)	-9.52	0.58					
Centered manipulated hatch	0.11	0.05					

Table S2: Broken stick analysis. Linear regression done with the 2 subsets of the data obtained after the broken stick analysis, that suggested regressions before and after the centred expected hatch date 5. Statistics are given for each term at the point of the exclusion of the term from the model. Estimates and standard errors are presented only for significant terms or main terms in the presence of significant interactions.

Response variable	Explanatory variables	Estimate	s.e.	ndf	ddf	R^2 (adjusted)	*F*-test	*p*-value
Moult-breeding overlap before 5	Centred expected hatch			1	60	0.44	29.80	**< 0.01 ***
	Treatment			2	60	0.44	5.57	**< 0.01 ***
	Centred expected hatch	-1.04	0.19					
	Treatment (Advanced)	4.14	1.53					
	Treatment (Control)	0.96	1.51					
	Treatment (Delayed)	-2.23	1.34					
Moult-breeding overlap after 5	Centred expected hatch			1	5	0.43	0.80	0.05
	Treatment			2	3	0.48	6.48	0.53

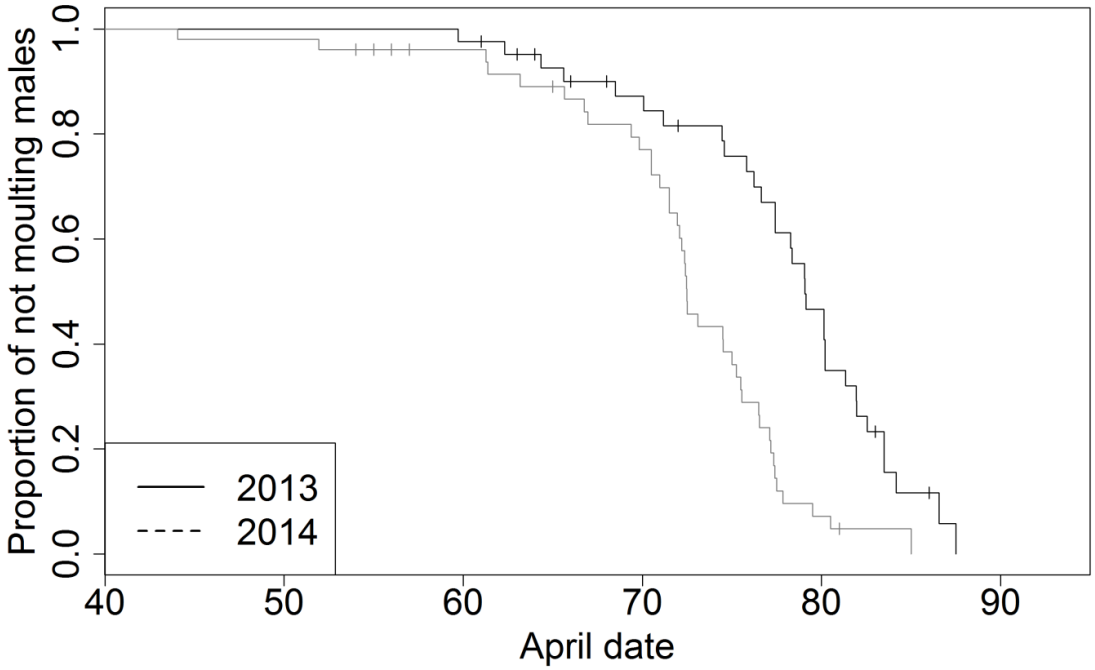

Figure S1: Proportion of males not moulting in relation to April date (days after 31st of March). Vertical lines in the graphs show the location of the censored data (non-moulting males when checked for the last time). Different lines on the graphs show the effects of year on the onset of moult.

Table S3: Adult condition. Results of the logistic regression analysis, explaining the probability of brood failure and analysis of count data explaining the number of 12 days old chicks in the nest; of the mixed effect models explaining the adult weight when chicks reached 12 days old.; of the linear regression explaining the variation in feather bending/stiffness and of the mixed effect model results explaining the number of visits to the brood per day. With the exception of feather quality and number of visit to the nest, analyses were done either with the expected hatching date (using laying date as a proxy) or manipulated hatching date (actual hatch date). Statistics are given for each term at the point of the exclusion of the term from the model. Estimates and standard errors are presented only for significant terms. Random effects for the model testing the number of visits are presented in Table S5.

1a) Probability of brood failure (expected hatch)	**Estimate**	**s.e.**	**df**	**Sample size**	**χ^2**	***p*-value**
Treatment : Centered expected hatch			2	103	1.72	0.42
Treatment			2	103	2.17	0.34
Centered expected hatch			1	103	2.13	0.14
Year			1	103	1.69	0.19
Start number of chicks			1	103	0.58	0.45

1b) Probability of brood failure (manipulated hatch)	**Estimate**	**s.e.**	**df**	**Sample size**	**χ^2**	***p*-value**
Treatment : Centered manipulated hatch			2	103	4.34	0.11
Treatment			1	103	3.88	0.14
Centered manipulated hatch			1	103	1.46	0.23
Year			1	103	1.69	0.19
Start number of chicks			1	103	0.52	0.47

2a) Number of 12 days old chicks (expected hatch)	**Estimate**	**s.e.**	**ndf**	**ddf**	**F-test**	***p*-value**
Treatment : Centered expected hatch^2			2	91	1.23	0.30
Treatment : Centered expected hatch			2	94	0.38	0.69
Centered expected hatch^2			1	93	0.14	0.71
Treatment			2	98	2.37	0.10
Centered expected hatch			1	97	0.61	0.44
Year			1	96	0.06	0.81
Start number of chicks	0.82	0.24	1	100	11.50	**<0.01***

2b) Number of 12 days old chicks (manipulated hatch)	**Estimate**	**s.e.**	**ndf**	**ddf**	**F-test**	***p*-value**
Treatment : Centered manipulated hatch^2			1	91	2.79	0.07
Treatment : Centered manipulated hatch			1	94	1.26	0.29
Centered manipulated hatch^2			1	93	1.37	0.25
Treatment			1	98	2.37	0.10
Centered manipulated hatch			1	97	0.35	0.55
Year			1	96	0.01	0.92
Start number of chicks	0.82	0.24	1	100	11.50	**<0.01***

3) Feather bending/stiffness	**Estimate**	**s.e.**	**ndf**	**ddf**	***F*-test**	***p*-value**
Treatment			2	8	0.35	0.72
Year			1	10	0.14	0.72

4a) Adult weight at day 12 (expected hatch)	Estimate	s.e.	ndf	ddf	*F*-test	*p*-value
Treatment : Sex : Year			3.00	80.39	3.60	**0.02***
Treatment (Advanced) : Sex (male) : Year (2014)	0.50	0.25				
Treatment (Control) : Sex (male) : Year (2014)	0.46	0.26				
Treatment (Delayed) : Sex (male) : Year (2014)	0.46	0.25				
Treatment (Control) : Sex (male)	0.22	0.27				
Treatment (Delayed) : Sex (male)	0.32	0.26				
Treatment (Control) : Year (2014)	0.41	0.28				
Treatment (Delayed) : Year (2014)	0.12	0.28				
Treatment (Advanced) : Centered expected hatch	0.00	0.01				
Treatment (Control) : Centered expected hatch	0.01	0.01				
Treatment (Delayed) : Centered expected hatch	0.00	0.02				
Treatment (Advanced)	12.45	0.14				
Treatment (Control)	12.04	0.15				
Treatment (Delayed)	12.13	0.14				
Sex (male)	-0.56	0.19				
Year (2014)	-0.31	0.19				

4b) Adult weight at day 12 (manipulated hatch)	Estimate	s.e.	ndf	ddf	*F*-test	*p*-value
Treatment : Sex : Year			3.00	80.42	3.61	**0.02***
Treatment (Advanced) : Sex (male) : Year (2014)	0.50	0.25				
Treatment (Control) : Sex (male) : Year (2014)	0.46	0.26				
Treatment (Delayed) : Sex (male) : Year (2014)	0.46	0.25				
Treatment (Control) : Sex (male)	0.22	0.27				
Treatment (Delayed) : Sex (male)	0.32	0.26				
Treatment (Control) : Year (2014)	0.41	0.28				
Treatment (Delayed) : Year (2014)	0.12	0.28				
Treatment (Advanced) : Centered manipulated hatch	0.00	0.01				
Treatment (Control) : Centered manipulated hatch	0.01	0.01				
Treatment (Delayed) : Centered manipulated hatch	0.00	0.01				
Treatment (Advanced)	12.45	0.15				
Treatment (Control)	12.04	0.15				
Treatment (Delayed)	12.15	0.14				
Sex (male)	-0.56	0.19				
Year (2014)	-0.31	0.19				

5a) Adult weight at day 12 - 2013 (expected hatch)	Estimate	s.e.	ndf	ddf	*F*-test	*p*-value
Treatment : expected hatch			2	41.59	0.44	0.65
Treatment : Sex			2	42.16	0.44	0.65
Treatment			2	42.71	1.81	0.18
expected hatch			1	44.25	0.07	0.79
Sex			1	44.83	9.21	**<0.01***
Sex (female)	12.22	0.09				
Sex (male)	11.85	0.09				

5b) Weight at day 12 - 2013 (manipulated hatch)	Estimate	s.e.	ndf	ddf	*F*-test	*p*-value
Treatment : manipulated hatch			2	41.97	0.34	0.71
Treatment : Sex			2	42.41	0.39	0.68
Treatment			2	42.71	1.81	0.18
manipulated hatch			1	45.90	0.00	0.96
Sex			1	44.83	9.21	**<0.01***
Sex (female)	12.22	0.09				
Sex (male)	11.85	0.09				

6a) Adult weight at day 12 - 2014 (expected hatch)	Estimate	s.e.	ndf	ddf	*F*-test	*p*-value
Treatment : expected hatch			2	46.32	0.28	0.75
Treatment : Sex			2	45.80	0.41	0.67
Treatment			2	49.18	0.40	0.68
expected hatch			1	47.93	0.00	0.98
Sex			1	47.81	1.39	0.24

6b) Adult weight at day 12 - 2014 (manipulated hatch)	Estimate	s.e.	ndf	ddf	*F*-test	*p*-value
Treatment : manipulated hatch			2	47.07	0.18	0.83
Treatment : Sex			2	45.79	0.41	0.67
Treatment			2	49.18	0.40	0.68
manipulated hatch			1	49.38	0.01	0.92
Sex			1	47.81	1.39	0.24

7) Number of visits	**Estimate**	**s.e.**	**ndf**	**ddf**	***F*-test**	***p*-value**
Treatment : Sex : Chick age : Year			10	130.40	1.38	0.20
Treatment : Sex : Year			2	69.87	0.23	0.80
Treatment : Sex : Chick age			4	142.52	1.54	0.20
Sex : Chick age			2	146.47	0.11	0.89
Treatment : Sex			2	54.90	0.27	0.76
Year : Chick age			2	146.50	0.63	0.53
Treatment : Year			2	2.79	1.48	0.36
Sex : Year			1	102.31	0.34	0.56
Treatment : Chick age			1	104.02	0.35	0.56
Year			2	12.22	0.19	0.83
Treatment			2	28.25	1.04	0.37
Chick age			2	155.35	3.07	0.05
Sex			1	71.26	13.62	**<0.01***
Sex (female)	360.88	11.66				
Sex (male)	297.92	12.44				

Table S4: Chick condition. Model results for the mixed effect models explaining fitness components of the 12 days old chicks. Statistics are given for each term at the point of the exclusion of the term from the model. Analyses were done either with the expected hatching date (using laying date as a proxy) or manipulated hatching date (actual hatch date). Estimates and standard errors are presented only for significant terms or main terms in the presence of significant interactions. In case of significant interactions with continuous terms and factors, we don't provide statistics for the continuous main effects. Random effects are presented in Table S5.

1a) Chick weight at 12 days old (expected hatch)	Estimate	s.e.	ndf	ddf	*F*-test	*p*-value
Treatment : Centered expected hatch : Year			2	113.65	0.81	0.45
Treatment : Centered expected hatch			2	117.02	2.71	0.07
Treatment : Year			2	86.63	1.14	0.32
Centered expected hatch : Year			1	136.29	0.28	0.60
Treatment			2	171.96	1.69	0.19
Centered expected hatch			1	214.17	0.21	0.65
Year			1	271.09	0.01	0.93
Tarsus length	0.07	0.01	1	315.40	42.95	**<0.01***

1b) Chick weight at 12 days old (manipulated hatch)	Estimate	s.e.	ndf	ddf	*F*-test	*p*-value
Treatment : Centered manipulated hatch : Year			2.00	151.41	6.08	**<0.01***
Treatment (Control) : Centered manipulated hatch : Year (2014)	0.45	0.13				
Treatment (Delayed) : Centered manipulated hatch : Year (2014)	0.22	0.16				
Treatment (Advanced) : Centered manipulated hatch	0.10	0.09				
Treatment (Control) : Centered manipulated hatch	-0.26	0.10				
Treatment (Delayed) : Centered manipulated hatch	-0.15	0.13				
Treatment (Control) : Year	0.53	0.63				
Treatment (Delayed) : Year	0.54	0.68				
Centered manipulated hatch : Year (2014)	-0.12	0.09				
Treatment (Advanced)	2.33	1.43				
Treatment (Control)	1.53	1.39				
Treatment (Delayed)	2.06	1.42				
Year (2014)	-0.45	0.53				
Tarsus length	0.06	0.01				

2b) Chick weight at 12 days old - 2013 (manipulated hatch)	Estimate	s.e.	ndf	ddf	*F*-test	*p*-value
Treatment : manipulated hatch			2	42.16	1.32	0.28
Treatment			2	44.74	0.40	0.67
manipulated hatch			1	48.32	1.10	0.30
Tarsus length	0.06	0.01	1	265.02	36.84	**<0.01***

3b) Chick weight at 12 days old - 2014 (manipulated hatch)	Estimate	s.e.	ndf	ddf	*F*-test	*p*-value
Treatment : manipulated hatch			2	43.82	1.44	0.25
Treatment			2	45.60	1.49	0.24
manipulated hatch			1	52.42	0.20	0.66
Tarsus length	0.06	0.01	1	195.03	23.15	**<0.01***

Table S5: Random effects. *p*-values and variance components for random effects given as absolute values and also as a percentage of the total variance. Analyses were done either with the expected hatching date (using laying date as a proxy) or manipulated hatching date (actual hatch date). P-values were obtained at the point of exclusion of each random effect from the minimal model (retaining the significant fixed effects shown in Tables S3 and S4).

	Expected hatch			Manipulated hatch		
1) Parental care	**Variance**	**Variance (%)**	**P-value**			
Random effect (Individual)	5665.00	75.66%	**<0.01***			
Random effect (Brood)	0.00	0.00%	1.00			
Residual	1822.00	24.34%				
Total	7487.00	100.00%				
2) Chick weight (at 12 days old)	**Variance**	**Variance (%)**	**P-value**	**Variance**	**Variance (%)**	**P-value**
Random effect (true parents)	0.14	7.14%	**<0.01***	0.11	5.76%	**<0.01***
Random effect (foster parents)	1.10	58.01%	**<0.01***	0.97	53.11%	**<0.01***
Residual	0.66	34.85%		0.75	41.14%	
Total	1.89	100.00%		1.83	100.00%	
2) Chick weight (at 12 days old) - 2013				**Variance**	**Variance (%)**	**P-value**
Random effect (true parents)				0.16	10.10%	0.05
Random effect (foster parents)				0.68	43.65%	**<0.01***
Residual				0.72	46.25%	
Total				1.57	100.00%	
3) Chick weight (at 12 days old) - 2014				**Variance**	**Variance (%)**	**P-value**
Random effect (true parents)				0.11	5.48%	**0.03***
Random effect (foster parents)				1.18	59.51%	**<0.01***
Residual				0.69	35.01%	
Total				1.98	100.00%	